ONE OF THE CONDITIONS UNDER WHICH DISCONTINUOUS STERILIZATION MAY BE INEFFECTIVE

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Since the process of sterilization by heat forms the foundation for all bacteriological work, we might take it for granted that this subject had been pretty well exhausted. New phases, however, appear with new kinds of work, and the discontinuous sterilization of large quantities of bouillon has been so unsatisfactory in my hands that I deem it worth while to present some facts upon this well-worn subject. Without doubt experiences similar to those related below have visited other laboratories, but thus far the causes have not been discussed. Text-books are, as a rule, silent upon the subject. Discontinuous heating at 100° C. is regarded as co-ordinate and interchangeable with the use of a more elevated temperature, and the whole subject is dismissed very briefly. Among a large number of text-books examined Abbott's Principles of Bacteriology was the only one found in which some qualifying statement is made in regard to this matter: "It must be borne in mind that this method of sterilization is only applicable in those cases which present conditions favorable to the germination of the spores into mature vegetative cells. Dry substances or organic materials in which decomposition is far advanced, where the conditions of nutrition favorable to the germination of spores are not present, cannot be successfully sterilized by the intermittent method."*

The general proposition as here stated includes the subject I wish to discuss. The illustrations given do not, however, strictly apply to the bacteriological laboratory, since dry or decomposed substances are rarely dealt with there. A more apt illustration is the condition to be described.

* Second edition, p. 53.

For more than a year past bouillon steamed in shallow layers (2 to 3 cm. deep) in the Arnold sterilizer for an hour on 3 or 4 successive days, though remaining indefinitely clear if unused, showed after some days' growth of diphtheria bacilli the presence of anaërobes. The source of the contamination was at first looked for in some error of manipulation during inoculation and in the stock culture employed. Soon, however, both these sources were excluded and the infection was referred to spores which had survived the boiling. It is interesting to note here that the bouillon became favorable to the germination of these spores and the multiplication of the bacilli only after the diphtheria bacilli had formed a membrane on the surface and were appropriating all the available oxygen. None of the flasks were ever found harboring aërobic spore-bearing bacilli.

Similar favoring conditions have been observed in the past on the surface of solid culture media when aërobes and anaërobes were introduced together. That the former may arouse slumbering spores of the latter lurking in culture media has not, I believe, been suggested, although the matter is self-evident. This experience is, as it were, an echo of the prolonged controversy which raged over the hypothesis of abiogenesis. Even at this day the acuteness of the intellects that finally solved the problem by satisfactory demonstrations is forcibly impressed upon the stumbling experimenter. In the case before us the conditions are somewhat different from those formulated by the participants in this controversy. We have a "putrescible" fluid which remains limpid. Inoculated with one bacillus, it apparently gives rise to another wholly different organism. The constancy of the second form in all the flasks examined, a large bacillus with a terminal spore, might in a more primitive state of our knowledge concerning bacteria have led to seemingly reasonable speculations concerning "the mutation of species" and the "spore-bearing phase of the diphtheria bacillus."

Discontinuous sterilization cannot therefore be relied upon when shallow layers of fluid freely exposed to the air are to be freed of bacteria. The spores of anaërobes accidentally present cannot find the conditions favorable to their germination in the intervals between

the successive applications of heat. The autoclave is therefore indicated, for a temperature of 110° to 115° C. definitely rids the fluid of them. In short, a bacteriological laboratory would be seriously crippled without apparatus furnishing temperatures above the boiling point. But by a round-about method discontinuous sterilization may be even here made successful.

Having cultivated anaërobes for a number of years without the use of hydrogen in vessels having a restricted communication with the air, I filled round litre flasks with bouillon to the neck and had them steamed three of four times and then placed in the incubator. In several instances they became suddenly turbid after 2 days by reason of the almost explosive rapidity with which anaërobic bacilli multiplied, thus proving the theory of imperfect sterilization of the bouillon used for the cultivation of diphtheria bacilli. Most of the flasks remained sterile after 4 steamings with intervals of incubation between them gradually lengthened to 48 hours. It is not to be denied that there may be still other anaërobes whose sensitiveness to aërobic conditions is so great that development may not take place even in the nearly full round flasks. In several instances bouillon prepared for the cultivation of tetanus bacilli became clouded with anaërobes after four or five steamings, indicating that even under favorable conditions spores of this character germinate with difficulty, and that not less than 48 hours of incubation should decide the sterility of the fluid. It is thus possible to eliminate anaërobes without the autoclave. If the bouillon is to be used in shallow layers, it must finally be poured or siphoned under suitable protection from the round flasks in which the sterilization was effected into the sterile culture flasks, but this round-about process will rarely be needed, since it does not appear that 110° to 115° C. is any more harmful than 100° C. to the nutritive quality of the bouillon.

This subject is of some importance in other respects. It is possible that now and then anaërobes present in tubes of milk, for instance, may remain dormant until some other organism is introduced. Subsequent changes in the milk due to the anaërobe may be ascribed to the organism introduced unless the sediment be carefully scrutinized

with the microscope. In tubes containing agar, gelatine, or other solid culture media, the restricted supply of oxygen in the bottom of the tubes will favor spore germination, so that the danger of subsequent contamination even when the autoclave is not resorted to is not likely to cause any trouble.

Sterilization from the point of view developed in these pages consists essentially in the destruction of all those bacteria or their spores which may subsequently find favorable conditions for development. The discovery by Globig* of very resistant spore forms and of bacteria which multiply only at a certain elevated temperature makes this definition accord with the facts. Inasmuch as our only test for the presence of life in any culture medium is its increase until visible mainly to the unaided eye, we are unable to affirm the non-existence of any life at all, although for practical purposes we may safely take it for granted after anaërobes have been destroyed.

The spores of adventitious anaërobes will probably be regarded as coming from contact of the beef with fecal matter at the abattoir—dust, dirt and utensils. There is, however, another source to which my attention has been called. In adding bits of tissue as large as peas from the organs of chloroformed rabbits and guinea-pigs, and in one case from the kidney of a pig slaughtered for food, to gelatine in tubes or to fermentation tubes pure cultures of anaërobes were obtained in four cases. It is thus not improbable that beef may contain a few stray spores which during the life of the animal have been carried from the digestive tube into different parts of the body, but which cannot germinate under prevailing conditions.

The various cultures thus far obtained from insufficiently sterilized bouillon and from animals have been reserved for further study and for possible identification with described forms. At present I may simply state that while they are all gas-producing bacilli bearing terminal spores, they evidently belong to different species or varieties of species, if we are to judge from the quite different odors and the variations in the gas formula $\frac{H}{CO_2}$ in dextrose bouillon as roughly determined in the fermentation tube.

* *Zeitschrift f. Hygiene*, iii (1887), 294, 322.